

D6 / 03

(11) (A) No. 1 128 418

(45) ISSUED 820727

(52) CLASS 167-139

(51) INT. CL. ³ A61K 39/395

(19) (CA) **CANADIAN PATENT** (12)

(54) PURIFICATION OF GAMMAGLOBULIN DERIVATIVE

(72) Funatsu, Akinobu;
Oyama, Shuzoh;
Akimoto, Yoshinori;
Ohashi, Komei,
Japan

(73) Granted to Juridical Foundation the
Chemo-Sero-Therapeutic Research Institute, Japan

(21) APPLICATION No. 341,901

(22) FILED 791213

(30) PRIORITY DATE Japan (4177/1979) 790117

No. OF CLAIMS 12

PURIFICATION OF GAMMAGLOBULIN DERIVATIVE

Abstract of the Disclosure

The invention provides a process for the production of a highly purified S-sulfonated gammaglobulin by treating an S-sulfonated gammaglobulin, obtained by sulfonating a conventional gammaglobulin, with an ion exchanger in a buffer solution for development, and thereby absorbing single molecular S-sulfonated gammaglobulin thereon, and then eluting the single molecular S-sulfonated gammaglobulin with a buffer solution for elution. This process can give the desired S-sulfonated gammaglobulin having a high content of single molecules and an extremely small anticomplementary activity (e.g. CH_{50} of 10% or less) and also an excellent shaking stability from the conventional gammaglobulin on an industrial scale.

FIG. 1

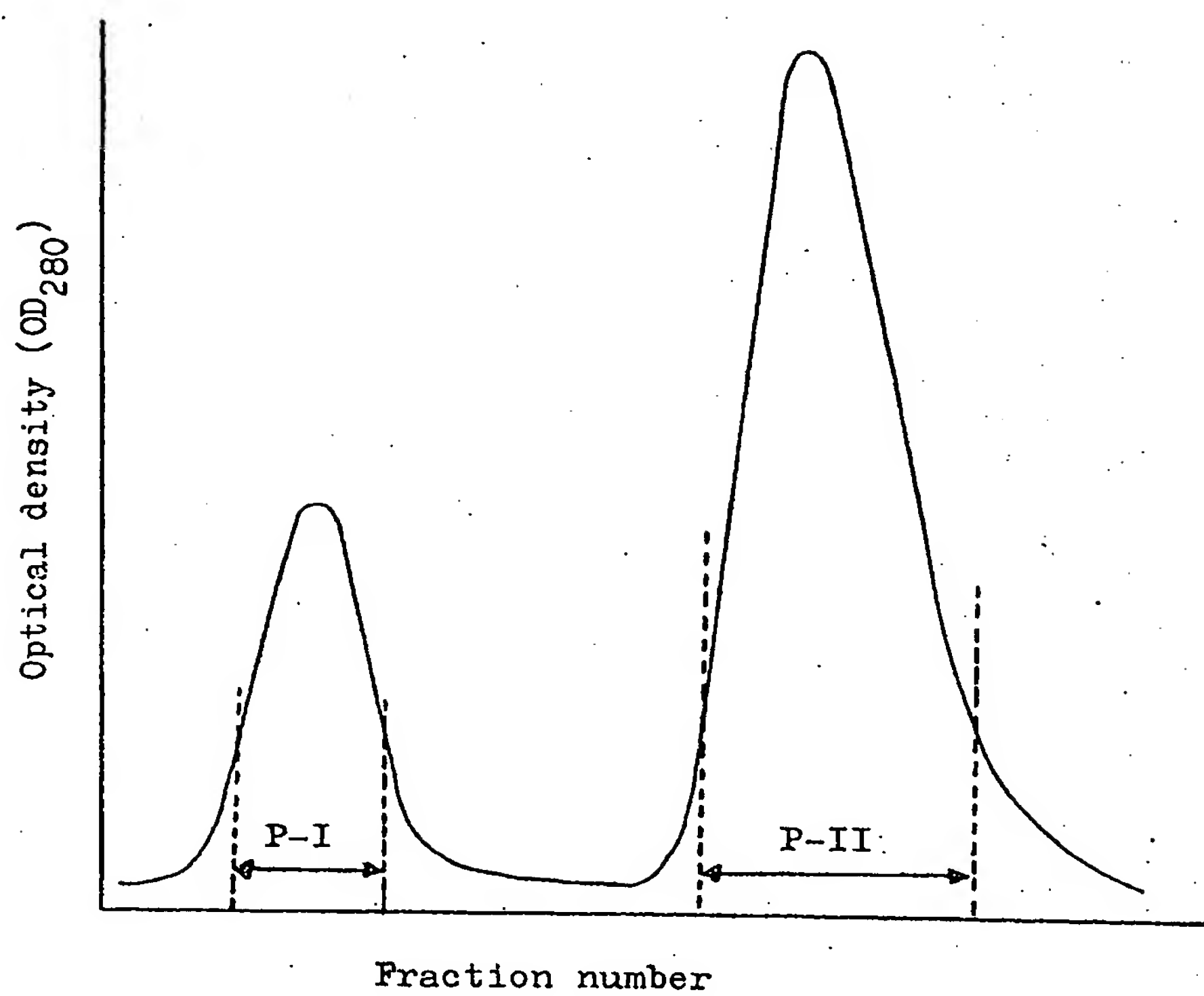
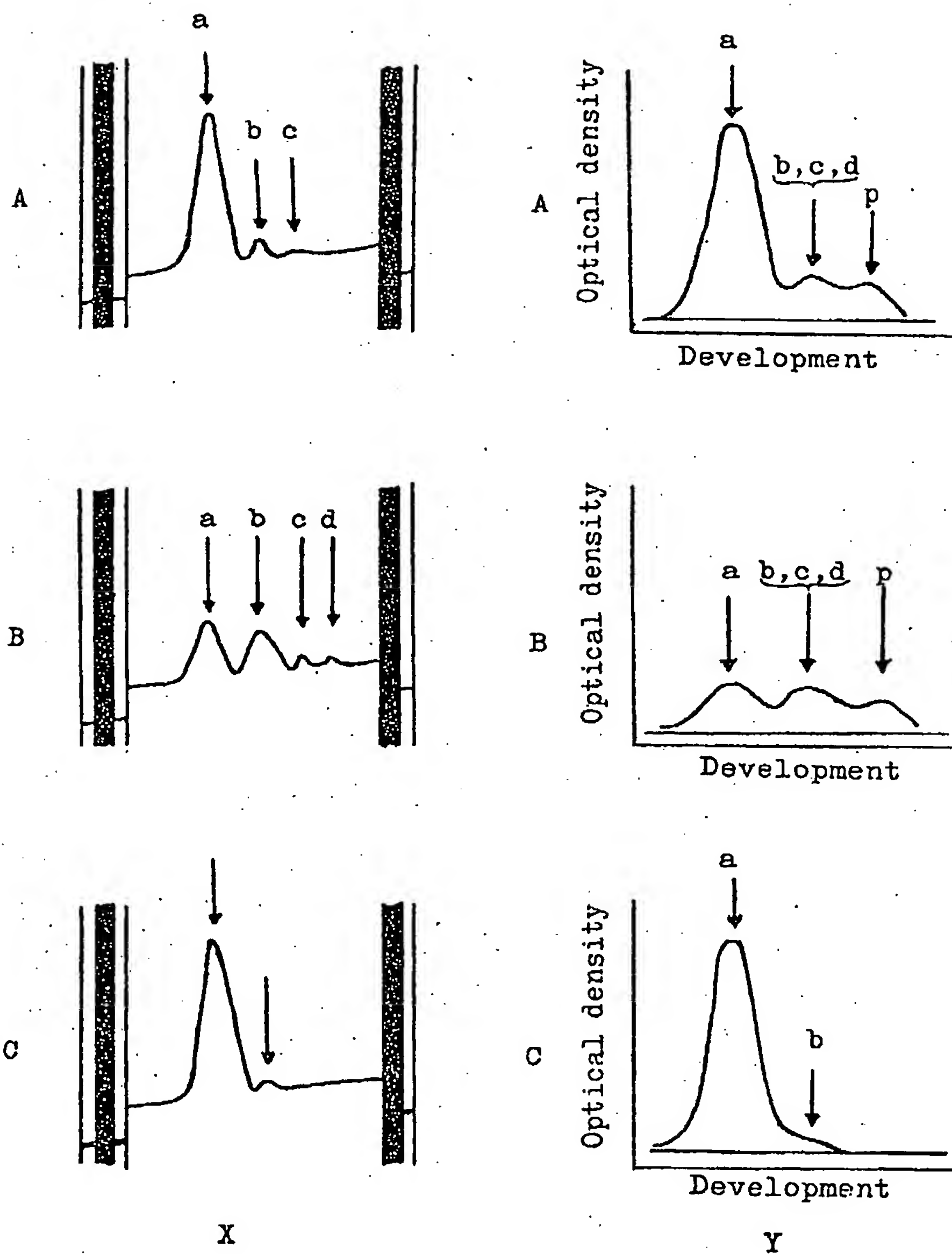


FIG. 2



The present invention relates to a method for purification of gammaglobulin. More particularly, it relates to a method for production of purified, S-sulfonated gammaglobulin.

It is well known that gammaglobulin fractionated from blood plasma has antibody activities against various infectious diseases and is useful as a so-called immunoglobulin preparation for the prophylactic and therapeutic treatment of various infectious diseases. However, conventional immunoglobulin preparations contain agglutinated molecules which are produced during the purification step thereof, and hence they can not be administered intravenously and can only be administered intramuscularly. It is also known that when a gammaglobulin preparation containing a large amount of agglutinated molecules is intravenously administered to a patient, a complement is rapidly activated in the patient, which induces various side effects such as lowering of blood pressure, increase of body temperature, disorders of the circulatory system, or the like. On the other hand, when the gammaglobulin is administered by an intramuscular route, the administration amount and the penetration rate of gammaglobulin into blood vessels are limited, which is not suitable when a rapid increase of the blood level of the antibody is required. Accordingly, there is a need to find an improved gammaglobulin which can be intravenously administered without causing anticomplementary activity.

Various methods have been proposed for the preparation of intravenously administrable gammaglobulin, for example, a method of treatment with pepsin [cf. H.E. Schultze; Deutsch Medizinische Wochenschrift, Vol. 87, page 1643

(1962)], and a method of the treatment with plasmin [cf. J.T. Sgouris; Vox Sanguinis, Vol. 13, page 71 (1967)].

However, in the method of treatment with a protease such as pepsin, the gammaglobulin is decomposed into two or more lower molecular weight compounds, and hence, the produced antibody disappears within a shorter period of time and further the biological activity of the Fc moiety of the gammaglobulin molecule is decreased since this moiety is cut off to a large extent.

10 It has also been proposed to produce an intravenously administrable gammaglobulin without the above drawbacks, i.e. to provide the desired gammaglobulin without substantially changing the structure of gammaglobulin, for example a method of treating gammaglobulin at pH 4 [cf. S. Barundun et al; Vox Sanguinis, Vol. 13, page 93 (1967)], and a method of treatment with β -propiolactone [cf. W. Stephan; Vox Sanguinis, Vol. 28, page 422 (1975)]. According to the treatment of pH 4, however, the content of agglutinated molecules is increased during the storage
20 of the gammaglobulin thus obtained and it tends to again increase the anticomplementary activity. Besides, the gammaglobulin produced by the treatment with β -propiolactone might possibly function as an antigen when administered.

It has recently been reported by Masuho et al that a suitable, intravenously administrable gammaglobulin can be produced by sulfonation of the S-S chain of gammaglobulin (cf. U.S. Patent 4,059,571, Japanese Patent Publication (unexamined) No. 1630/1976). The S-sulfonated gamma-
30 globulin produced by Masuho et al retains the original large molecule owing to hydrogen bonding while the S-S

bond is broken, and hence it shows remarkably decreased anticomplementary activity while completely retaining the antibody activity (they say that the anticomplementary activity level at a concentration of proteins of 5 % by weight [hereinafter referred to as " CH_{50} "] is 30 % or less, and further, the S-sulfonated gammaglobulin is readily reduced and then oxidized to produce the original gammaglobulin when administered to the human body [cf. Masuho et al, Journal of Biochemistry, Vol. 19, page 1377 (1976)]]. It was also proved that the S-sulfonated gamma-
10 globulin can be administered stably to low gammaglobulinemic or agammaglobulinemic subjects by clinical tests (cf. Noboru Kobayashi, International Academy of Blood Transfusion (Paris), 1978).

Thus, the S-sulfonated gammaglobulin is excellent and useful as an intravenously administrable gammaglobulin preparation and attention is given thereto. However, this product has a drawback that it is difficult to produce on an industrial scale. That is, according to the process
20 for the production thereof by Masuho et al as mentioned hereinbefore, when the starting gammaglobulin is sufficiently purified and has less agglutinated molecules, the desired S-sulfonated gammaglobulin having a low anticomplementary activity can be obtained, but when the conventional gammaglobulin which is used industrially as the starting material, for example the gammaglobulin produced by Cohn's fractionation method using ethanol at a low temperature, the produced S-sulfonated gammaglobulin has an anticomplementary activity which is not so low (at
30 the lowest CH_{50} = about 30 to 20 %). Thus, the process of Masuho et al is excellent in principle, but it should

be accompanied by an additional treatment in order to obtain the desired product having a high stability on an industrial scale.

Under these circumstances, the present inventors have made intensive studies to find an improved process for the production of the desired S-sulfonated gammaglobulin having a sufficiently low anticomplementary activity even when conventional gammaglobulin containing a large proportion of agglutinated molecules is used as the starting material. As a result, it has now been found that after the sulfonation reaction, the resulting S-sulfonated gammaglobulin is treated with an ion exchanger, and thereby, the desired S-sulfonated gammaglobulin having an extremely low anticomplementary activity can be obtained.

Thus, an object of the present invention is to provide an improved process for the production of an intravenously administrable gammaglobulin on an industrial scale.

According to the present invention, there is provided a process for the production of a purified S-sulfonated gammaglobulin, which comprises treating an S-sulfonated gammaglobulin with an ion exchanger in a buffer solution for development and thereby absorbing single molecular S-sulfonated gammaglobulin onto the ion exchanger, and then eluting the single molecular S-sulfonated gammaglobulin with a buffer solution for elution.

The single molecular S-sulfonated gammaglobulin obtained by the process of the present invention, at least in the preferred forms, has a CH₅₀ of less than 10 % and hence is suitable as an intravenously administrable immunoglobulin preparation.

The starting material for the S-sulfonated

gammaglobulin may be a conventional gammaglobulin, e.g. one prepared by Cohn's fractionation method which has been used internationally for the preparation of gammaglobulin [cf. J. Am. Chem. Soc., Vol. 68, page 459 (1946)]. The gammaglobulin is sulfonated by treating it with an oxidizing agent, e.g. an alkali metal tetrathionate, an alkali metal iodobenzoate, a molecular oxygen-containing gas (e.g. air) or a sulfite ion-generating compound (e.g. sulfurous acid) (cf. U.S. Patent 4,059,571, Japanese Patent Publication (unexamined) Nos. 1630/1976 and 76418/1976). The S-sulfonated gammaglobulin may optionally be purified by a conventional method, e.g. dialysis.

The ion exchanger used in the present invention is preferably a repeatedly usable column without specific activation having a large binding capacity and is preferably an autoclavable gel having good stability under various conditions, e.g. at various pH levels, ionic strengths, etc.

The ion exchanger includes anion exchangers and cation exchangers, but anion exchangers are preferable from the viewpoint of the biological and physical stabilities of the product. An anion exchanger may be used in combination with a cation exchanger.

Suitable examples of the anion exchanger are agarose gel introduced with an anionic substituent (e.g. DEAE-Sepharose CL-6B*), dextran gel introduced with an anionic substituent (e.g. DEAE-Sephadex*, QAE-Sephadex*), cellulose gel introduced with an anionic substituent (e.g. DEAE-cellulose*, TEAE-cellulose*), polyvinyl gel introduced with an anionic substituent (e.g. DEAE-TOYOPEAL*),

* Trade Mark

amylose gel introduced with an anionic substituent, or the like. The anionic substituent may be, for example, diethylaminoethyl (DEAE), triethylaminoethyl (TEAE) and diethyl-(2-hydroxypropyl)aminoethyl(QAE).

Suitable examples of the cation exchanger are agarose gel introduced with a cationic substituent (e.g. CM-Sepharose CL-6B*), dextran gel introduced with a cationic substituent (e.g. CM-sephadex*, SP-Sephadex*), cellulose gel introduced with a cationic substituent (e.g. CM-cellulose*), polyvinyl gel introduced with a cationic substituent (e.g. CM-TOYOPEAL*), amylose gel introduced with a cationic substituent, or the like. The cationic substituent may be, for example, carboxymethyl (CM), sulfopropyl (SP) and sulfoethyl (SE).

The absorption of the S-sulfonated gammaglobulin onto an ion exchanger may be carried out as follows. The S-sulfonated gammaglobulin is treated with an ion exchanger in a buffer solution for development which has an optimum hydrogen ion level (pH level) and an optimum ionic strength, and thereby, the single molecular S-sulfonated gammaglobulin is absorbed onto the ion exchanger. By this treatment, the agglutinated molecules of S-sulfonated gammaglobulin and also un-sulfonated gammaglobulin pass through the ion exchanger without being absorbed thereon. After the absorption, the ion exchanger is washed with the same buffer solution in order to completely remove the agglutinated gammaglobulin and other impurities.

The buffer solution used for the absorption of the single molecular S-sulfonated gammaglobulin preferably has pH level of 4 to 10, desirably 7 to 8, and an ionic

* Trade Mark

strength (μ) of 0.01 to 0.15, desirably 0.03 to 0.09, in the case of anion exchanger. When a cation exchanger is used, the buffer solution preferably has a pH level of 4 to 6.5, desirably 5 to 6, and an ionic strength of 0.01 to 0.1, desirably 0.035 to 0.07. The concentration of the S-sulfonated gammaglobulin to be subjected to the absorption treatment is not critical, but in view of the exchange capacity of the ion exchanger, the S-sulfonated gammaglobulin is preferably used in a concentration of 2 to 12 W/V %.

The buffer solution for the development includes a phosphate buffer solution, a citrate buffer solution, a Tris-phosphate buffer solution, a Tris-HCl buffer solution, a borate buffer solution, an acetate buffer solution, or the like.

The single molecular S-sulfonated gammaglobulin absorbed onto the ion exchanger is easily recovered therefrom by eluting out with a buffer solution which has a pH level and ionic strength different from those of the buffer solution for development. The buffer solution for elution has a pH level of 3 to 4 when using an anion exchanger and a pH level of 6 to 9 when using a cation exchanger. The ionic strength (μ) of the buffer solution for elution should be higher than that of the buffer solution for development and it is preferably in the range of 0.05 to 0.8. The buffer solution for elution may be, for example, a phosphate buffer solution, a glycine-HCl buffer solution, a citrate buffer solution, an aqueous solution of sodium acetate, or the like. These buffer solutions may contain sodium chloride.

The purification treatment of the present invention is

usually carried out at room temperature, but may be done under cooling.

The S-sulfonated gammaglobulin purified by the present invention has a higher content of single molecules, a less anticomplementary activity and a greater stability in comparison with the product before purification. For instance, when the S-sulfonated gammaglobulin as used in Example 1 hereinafter (three lots) was purified according to the process of the present invention using DEAE-
10 Sepharose CL-6B and CM-Sepharose CL-6B and the resulting product was regulated so as to have a protein concentration of 5 %, the products showed such an anticomplementary activity (CH_{50}), content of single molecule (measured by an ultra-centrifugal analysis) and shaking stability (measured by means of the difference of light-scattering after shaking with Kahn's shaking machine) as shown in Table 1.

1128418

Table 1

	Lot No.	Before purification	After purification	
			Using DEAE-Sephadex	Using CM-Sephadex
Anticomplementary activity (CH ₅₀) (%)	i	25	6	8
	ii	20	3	10
	iii	22	5	7
Content of single molecule (%)	i	82	92	90
	ii	84	92	92
	iii	85	94	95
Shaking stability	i	100	5	42
	ii	80	8	38
	iii	136	2	30

[Remarks]: *1) This was measured according to the Kabat Mayer process [cf. Experimental Immunochimistry page 225 (1961)].

*2) This was measured after centrifugation at 60,000 rpm for 50 minutes with a Beckmann ultra-centrifugal machine.

*3) This was measured by shaking the product at an amplitude of 3.4 cm/sec. and 3.7 cycle/sec. for 4 hours, irradiating with a light and then measuring the difference of the light-scattering before and after the shaking (cf. Standard for Biological Preparations, editing by Ministry of Health and Welfare, Japan).

As is clear from the results as shown in Table 1, the S-sulfonated gammaglobulin purified by the present invention shows an extremely decreased anticomplementary activity, i.e. 10 % or less at a protein concentration of 5 %, in comparison with that of the product before

purification. When using an anion exchanger, it is particularly decreased. The content of single molecules is increased from 75 to 80 % (before purification) to 90 to 95 % (after purification). According to ultracentrifugal analysis, the largely agglutinated molecules are difficult to analyze, because they precipitate immediately after initiation of centrifugation, but according to gel chromatographic analysis (e.g. thin layer gel chromatography), the agglutinated molecules can be separated into polymers and oligomers. According to this gel chromatographic analysis, it was confirmed that the content of monomer (single molecule) was increased from 60 to 70% (before purification) to 85% or more (after purification). Moreover, according to the measurement of light-scattering before and after purification, the S-sulfonated gammaglobulin purified by the present invention shows a high stability and no insoluble substance precipitates even by shaking.

The present invention is illustrated by the following Examples, wherein % means % by weight unless otherwise specified.

Reference is made in these Examples to the accompanying drawings, in which:

Fig. 1 is a graph showing the separation pattern of fractions produced according to one of the Examples; and

Fig. 2 is a collection of graphs showing the results of ultra centrifugal analysis of fractions before and after treatment as in the invention.

Example 1

Sodium tetrathionate (248g) and sodium sulfite (408g) were separately dissolved in a sodium chloride-containing

1128418

phosphate buffer solution (pH 7.6) (1,500 ml and 3,500 ml, respectively), followed by filtration for sterilization. Each solution thus prepared was added to a 15 % aqueous solution of gammaglobulin (10 liters) which was prepared from human blood plasma by the ethanol-fractionation method and the mixture was slowly stirred at 43°C for 4.5 hours to cause sulfonation.

10 After the reaction, the reaction mixture was dialyzed against a physiological saline solution in order to remove the excess sulfonating agent and then equilibrated with a buffer solution for development (a phosphate buffer solution; $\mu = 0.06$, pH 7.5).

20 The solution of S-sulfonated gammaglobulin in phosphate buffer was regulated to provide a protein concentration of about 8 %, and the solution (15 liters) was developed by passing it through a column (16 liters) packed with DEAE-Sephadex CL-6B (Trade Mark made by Pharmacia) which was equilibrated with the same phosphate buffer solution as used above. The fraction (P-I) which passed through the column without being absorbed onto the ion exchanger was a solution having a protein concentration of about 2 % (16 liters).

The column was washed well with the same phosphate buffer solution as used above, and when almost no further protein was detected, an acetate buffer solution ($\mu = 0.1$, pH 4.0) was passed through the column and the eluted fraction (P-II) was collected. Said fraction (P-II) was a solution having a protein concentration of about 2.5 % (32 liters).

30 Each fraction obtained above was neutralized with an aqueous sodium hydroxide solution, concentrated until the

1128418

fraction at a wave length of 280 nm.

Besides, the solution of S-sulfonated gammaglobulin before purification (A), the P-I fraction (B) and the P-II fraction (C) were subjected to ultra-centrifugal analysis (X) and thin layer gel filtration analysis (Y). The patterns in these analyses are shown in the accompanying Figure 2. The ultra-centrifugal analysis was carried out under the conditions of a protein concentration of about 1.67 %, at 60,000 rpm for 50 minutes by using a Beckmann
10 ultra-centrifugal machine, and the thin layer gel filtration analysis was carried out in accordance with Migita's method [cf. Annual Report of Inst. Virus Research, Kyoto Univ., Vol. 8, page 130 (1965)]. In Figure 2, "a" is a single molecular substance (7S), and "b", "c" and "d" are oligomers (9S, 11S and 13S, respectively), and "p" is a polymer. As is clear from Figure 2, both analyses show similar patterns, but the amount of the largely agglutinated molecules appears higher in the thin layer gel filtration analysis than in
20 the ultra-centrifugal analysis. In the pattern of the ultra-centrifugal analysis, the polymer does not appear. Both analyses show that the P-I fraction contains a large amount of agglutinated molecules and the P-II fraction contains an increased amount of single molecule.

Example 2

A 15 % aqueous solution of gammaglobulin was sulfonated and subjected to dialysis in the same manner as described in Example 1. The S-sulfonated gammaglobulin solution thus obtained was regulated so as to provide a
30 protein concentration of about 7 %. The solution (about 100 liters) was passed through a column (150 liters)

protein concentration became about 5 %, and then dialyzed against a 2.25 % glycine-containing isotonic phosphate buffer solution. Various properties of the products thus obtained were measured in the same manner as shown in Table 1. The results are shown in Table 2.

Table 2

	Before purification	After purification	
		P-I	P-II
Anticomplementary activity (CH ₅₀) (%)	24	52	4
Content of single molecule (%)	82	50	94
Shaking stability	120	207	3

As is clear from the above results, the P-I fraction has a high anticomplementary activity and contains a large amount of agglutinated molecules and is inferior in shaking stability. On the other hand, the desired P-II fraction has a sufficiently low anticomplementary activity, a high content of single molecules and an extremely increased shaking stability. Thus, the components having undesirable properties are effectively removed as the P-I fraction.

The separation pattern of the fractions in the above Example 1 is shown in the accompanying Figure 1, wherein the abscissa represents the fraction number and the ordinate axis represents the optical density of each

1128418

packed with DEAE-Sepharose CL-6B (Trade Mark) which was equilibrated with a citrate buffer solution ($\mu = 0.03$, pH 7.5). The fraction (P-I) which passed through the column without being absorbed onto the ion exchanger was a solution having a protein concentration of about 1.5 % (about 125 liters).

After washing the column in the same manner as described in Example 1, a sodium chloride-containing glycine-HCl buffer solution ($\mu = 0.55$, pH 3.5) was passed through the column and the eluted fraction (P-II) was collected. Said fraction (P-II) was a solution having a protein concentration of about 2 % (about 245 liters).

The fractions thus obtained were treated in the same manner as described in Example 1, and the various properties thereof were measured likewise. The results are shown in Table 3.

Table 3

	Before purification	After purification	
		P-I	P-II
Anticomplementary activity (CH_{50}) (%)	25	58	5
Content of single molecule (%)	82	50	94
Shaking stability	137	238	3

1128418

Example 3

In the same manner as described in Example 1, except that a citrate buffer solution ($\mu = 0.03$, pH 7.5) was used instead of the phosphate buffer solution ($\mu = 0.06$, pH 7.5) as the buffer solution for development, a solution (about 15 liters) of S-sulfonated gammaglobulin having a protein concentration of about 7 % was developed by passing it through a column (16 liters) which was packed with DEAE-Sepharose CL-6B (Trade Mark). As a result, a P-I
10 fraction (15.6 liters) was obtained having a protein concentration of about 1.7 %. By using a citrate buffer solution ($\mu = 0.614$, pH 6.0) as the buffer solution for elution, a P-II fraction (31 liters) was obtained having a protein concentration of about 2.2 %.

Example 4

The above Example 3 was repeated except that a phosphate buffer solution ($\mu = 0.06$, pH 7.5) was used. As a result, a P-I fraction (about 14 liters) was obtained having a protein concentration of about 1.8 %. Further-
20 more, by elution with a glycine-HCl buffer solution ($\mu = 0.542$, pH 3.5), a P-II fraction (29 liters) was obtained having a protein concentration of about 2.4 %.

Example 5

The above Example 3 was repeated except that a Tris-HCl buffer solution ($\mu = 0.0175$, pH 8.5) was used as the buffer solution for development and a sodium chloride-containing phosphate buffer solution ($\mu = 0.45$, pH 6.0) was used as the buffer solution for elution. As a result, a P-I fraction (22 liters) having a protein
30 concentration of about 1.2 % and a P-II fraction (27 liters) having a protein concentration of about 2.6 % were

obtained.

Example 6

An aqueous solution of S-sulfonated gammaglobulin prepared in the same manner as described in Example 1 was equilibrated by dialysis against an acetate buffer ($\mu = 0.05$, pH 5.3). The solution was regulated so as to provide a concentration of S-sulfonated gammaglobulin of about 7 %, and the resulting solution (15 liters) was passed through a column (16 liters) packed with
10 CM-Sepharose CL-6B (Trade Mark made by Pharmacia) which was equilibrated with the same acetate buffer solution as used above. The fraction (P-I) which passed through the column without being absorbed thereon was a solution having a protein concentration of about 1.9 % (13 liters).

After washing the ion exchanger column in the same manner as described in Example 1, an aqueous solution of sodium acetate ($\mu = 0.2$) was passed through the column and the eluted fraction (P-II) was collected. Said P-II
20 fraction was a solution having a protein concentration of about 2.5 % (27 liters).

Each fraction thus obtained was treated in the same manner as described in Example 1, and various properties thereof were measured likewise. The results are shown in Table 4.

1128413

Table 4

	Before purifi- cation	After purification	
		P-I	P-II
Anticomple- mentary activity (CH ₅₀) (%)	25	32	8
Content of single molecule (%)	83	48	95
Shaking stability	102	180	32

10

1128413

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A process for the production of a purified S-sulfonated gammaglobulin, which comprises treating an S-sulfonated gammaglobulin with an ion exchanger in a buffer solution for development and thereby absorbing single molecular S-sulfonated gammaglobulin onto the ion exchanger, and then eluting the single molecular S-sulfonated gamma-globulin with a buffer solution for elution.
2. A process according to claim 1, wherein the ion exchanger is an anion exchanger.
3. A process according to claim 2, wherein the anion exchanger is a member selected from the group consisting of agarose gel introduced with an anionic substituent, dextran gel introduced with an anionic substituent, cellulose gel introduced with an anionic substituent, polyvinyl gel introduced with an anionic substituent, and amylose gel introduced with an anionic substituent, said anionic substituent being a member selected from the group consisting of diethylaminoethyl, triethylaminoethyl and diethyl-(2-hydroxypropyl)aminoethyl.
4. A process according to claim 2, wherein a buffer solution having a pH level of 4 to 10 and an ionic strength of 0.01 to 0.15 is used as the buffer solution for development.
5. A process according to claim 2, wherein a buffer solution having a pH level of 3 to 4 and an ionic strength of higher than that of the buffer solution for development is used as the buffer solution for elution.
6. A process according to claim 5, wherein the ionic strength of the buffer solution for elution is in the

range of 0.05 to 0.8.

7. A process according to claim 1, wherein the ion exchanger is a cation exchanger.
8. A process according to claim 7, wherein the cation exchanger is a member selected from the group consisting of agarose gel introduced with a cationic substituent, dextran gel introduced with a cationic substituent, cellulose gel introduced with a cationic substituent, polyvinyl gel introduced with a cationic substituent, and amylose gel introduced with a cationic substituent, said cationic substituent being a member selected from the group consisting of carboxymethyl, sulfopropyl and sulfoethyl.
9. A process according to claim 7, wherein a buffer solution having a pH level of 4 to 6.5 and an ionic strength of 0.01 to 0.1 is used as the buffer solution for development.
10. A process according to claim 7, wherein a buffer solution having a pH level of 6 to 9 and an ionic strength of higher than that of the buffer solution for development is used as the buffer solution for elution.
11. A process according to claim 10, wherein the ionic strength of the buffer solution for development is in the range of 0.05 to 0.8.
12. A purified S-sulfonated gammaglobulin having a high content of single molecules and a small anticomplementary activity, which is produced by the process as set forth in claim 1.



**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.